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(74) Agent: GEIGER, Kathleen, W.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

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(71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

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(54) Title: SCORPION TOXINS

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SEQ ID NO: 21 -----EHGYLLNKYTG [C] KVM [C] VINNEE [C] GYL [C] NKRRGGYGY [C] YF  
SEQ ID NO: 02 MMKIIIFLIVSSLMLIGVKSDGYLLNKATG [C] KVM [C] VVNNAS [C] NSS [C] -KLRGGNYGY [C] YF  
1 60

\*\*\*\*\*  
SEQ ID NO: 21 WKLA [C] Y [C] QGARKSELWNYKTNK [C] D--L  
SEQ ID NO: 02 WKLA [C] Y [C] EGVAKSELWAYETNT [C] NGKL  
61 87

(57) Abstract: This invention relates to an isolated nucleic acid fragment encoding scorpion toxins that are Na-channel modifiers. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the Na-channel modifier, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the Na-channel modifier in a transformed host cell.

TITLE

## SCORPION TOXINS

This application claims the benefit of U.S. Provisional Application No. 60/140,410, filed June 22, 1999.

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FIELD OF THE INVENTION

This invention is in the field of molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding scorpion toxins that are sodium channel modifiers.

BACKGROUND OF THE INVENTION

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Alpha neurotoxins are short, single-chain, polypeptides responsible for insect and mammal poisonings. These neurotoxins show variability in their apparent toxicity; in their primary structures, and in their binding features to neuronal membrane preparations (Dufton and Rochat (1984) *J. Mol. Evol.* 20:120-127). Despite differences in their primary structures and phylogenetic selectivity, scorpion neurotoxins affecting sodium (Na) channels are closely related in their spatial arrangements and form a compact globular structure kept rigid by the four disulfide bridges (Miranda et al. (1970) *Eur. J. Biochem.* 16:514-523; and Fontecilla-Camps (1989) *J. Mol. Evol.* 29:63-67).

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Zilbergberg and coworkers determined that single amino acid residues are important for receptor binding and for biological activity of scorpion Na-channel toxins (Zilbergberg et al. (1997) *J. Biol. Chem.* 272:14810-14816). As examples, the lysine at position 8 of LqhIT was demonstrated to be necessary for binding activity and toxicity without change in overall structure. A substantial decrease in biological activity without a significant change in structure was found when the aromatic amino acid phenylalanine, at position 17, was substituted for glycine. Conversely, changes in structure are not necessarily associated with differences in toxicity as demonstrated when tyrosine at position 49 was changed to leucine.

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While potassium (K) channels have been shown to be central to heart function, the role of chlorine- (Cl) and Na-channels in this activity is less clear (Johnson et al. (1998) *J. Neurogent.* 12:1-24). Sodium entry hyperpolarizes the cell, producing indirect, Na-dependent changes of calcium transport (Friedman (1998) *Annu. Rev. Physiol.* 60:179-197). Abnormal influx of calcium is thought to be very important in the pathogenesis of several central nervous system disorders in vertebrates, including stroke damage, epilepsy, and the neuronal death associated with chronic epilepsy.

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Excitatory amino acids, most notably glutamate and aspartate, are the predominant excitatory neurotransmitters in the vertebrate (including human) central nervous system. These amino acids are released from presynaptic nerve terminals and, after diffusing across the synaptic cleft, contact special receptor molecules in the postsynaptic cell membrane. These receptors indirectly influence the flow of various ions across the cell membrane and thus contribute to production of an electrical response to the chemical message delivered by

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neurotransmitter molecules. A number of common and very serious neurological problems involve the abnormal functioning of excitatory amino acid synapses. These include epilepsy, several degenerative disorders such as Huntington's disease, and neuronal death following stroke. Unfortunately, there are very few chemical agents which are potent and selective  
5 blockers of excitatory amino acid receptors. Na-channel modifiers may be used for these purposes.

A drug with high affinity for the receptor could be expected to produce irreversible blockade of synaptic transmission. When labeled with some tracer molecule, such a drug would provide a reliable way of tagging receptors to permit measurement of their number  
10 and distribution within cells and tissues. These features would have very valuable consequences for research on excitatory amino acid neurotransmission and for the development of therapeutic agents to treat central nervous system dysfunction in humans and animals. Methods for treating heart and neurological diseases by applying toxins derived from spiders have been described (U.S. Patent No. 4,925,664).

15 Arthropod animals, including insects, and certain parasitic worms use excitatory amino acids as a major chemical neurotransmitter at their neuromuscular junction and in their central nervous system. Because of the damage done by insect pests and the prevalence of parasitic worm infections in animals and humans in many countries, there is a constant need for potent and specific new pesticides and anthelmintic drugs that are non-toxic to humans,  
20 pets, and farm animals.

Chemical insecticides are an integral component of modern agriculture, and are an effective means for reducing crop damage by controlling insect pests. However, chemical agents are under continuous scrutiny due to the potential for environmental contamination, selection of resistant populations of agronomic pests, and toxicity to non-target organisms  
25 such as beneficial insects, aquatic organisms, animals and humans. As a result, alternative strategies for insect control are being sought that are effective and yet benign to non-target populations and the environment. One of these strategies is to use microorganisms that are naturally occurring pathogens of target insect populations. The expression of scorpion toxins using baculovirus vectors will be an advantage since these toxins have been previously  
30 shown to be highly toxic and very specific (Zlotkin et al. (1995) American Chemical Society, Symposium on Agrochemicals).

Due to a combination of problems associated with some synthetic insecticides, including toxicity, environmental hazards, and loss of efficacy due to resistance, there exists a continuing need for the development of novel means of invertebrate control, including the  
35 development of genetically engineered recombinant baculoviruses which express protein toxins capable of incapacitating the host more rapidly than the baculovirus infection per se.

Scorpion venoms have been identified as possible sources of compounds providing insecticidal properties. Two insect-selective toxins isolated from the venom of the scorpion

*Leiurus quinquestriatus* and affecting sodium conductance have been reported previously (Zlotkin et al. (1985) *Arch. Biochem. Biophys.* 240:877-87). One toxin, AaIT, induced fast excitatory contractive paralysis of fly larvae and the other, LqhIT2, induced slow depressant flaccid paralysis suggesting that these two toxins have different chemical and pharmacological properties (Zlotkin et al. (1971) *Biochimie* (Paris), 53:1073-1078). Thus, other toxins derived from scorpion venom will also have different chemical and pharmacological properties.

#### SUMMARY OF THE INVENTION

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence of at least 180 nucleotides selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36; (b) a second nucleotide sequence encoding a polypeptide of at least 60 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 29, 31, 33, 35, and 37; or (c) a third nucleotide sequence comprising the complement of the first or second nucleotide sequences.

In a second embodiment, this invention relates to an isolated polynucleotide encoding a mature scorpion Na-channel agonist.

In a third embodiment, this invention concerns an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36 and the complement of such nucleotide sequences.

In a fourth embodiment, this invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to at least one suitable regulatory sequence.

In a fifth embodiment, the present invention concerns a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, an insect cell or mammalian cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

In a sixth embodiment, the invention also relates to a process for producing a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting a compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

In a seventh embodiment, the invention concerns a scorpion Na-channel agonist polypeptide of at least 60 amino acids comprising at least 80% identity based on the Clustal

method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 29, 31, 33, 35, and 37.

In an eighth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of a scorpion Na-channel agonist polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of a scorpion Na-channel agonist amino acid sequence.

In a tenth embodiment, this invention relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a scorpion Na-channel agonist polypeptide comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing a cDNA or genomic fragment that comprises the isolated DNA clone.

In an eleventh embodiment, this invention concerns a composition, such as a hybridization mixture, comprising an isolated polynucleotide or an isolated polypeptide of the present invention.

In a twelfth embodiment, this invention concerns a method for expressing a gene encoding Na-channel agonist in the genome of a recombinant baculovirus in an insect cell culture or in viable insects wherein said insect cells or insects have been genetically engineered to express an AAH IT4, an LqhIT2 precursor, an IT-2 precursor, a TsnTxp, an insecticidal toxin, a BmK M1 precursor, a neurotoxin V-5, or an AS neurotoxin. The recombinant baculovirus expression vector comprising a DNA sequence encoding a polypeptide of at least 60 amino acids comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 29, 31, 33, 35 and 37.

#### BRIEF DESCRIPTION OF THE

#### DRAWINGS AND SEQUENCE LISTING

The invention can be more fully understood from the following detailed description, the accompanying drawings and the Sequence Listing which form a part of this application.

Figure 1 depicts the alignment between the AAH IT4 amino acid sequence from *Androctonus australis hector* (NCBI General Identifier No. 134360; SEQ ID NO:21) and the amino acid sequence derived from the instant *Buthotus judaicus* clone ibj1c.pk008.19 (SEQ ID NO:2). The top row indicates with asterisks (\*) the amino acids conserved in both

sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 2 depicts the alignment between the LqhIT2 precursor amino acid sequence from *Leiurus quinquestriatus hebraeus* (NCBI General Identifier No. 1078960; SEQ ID NO:22) and the amino acid sequence derived from the instant *Buthotus judaicus* clone ibjlc.pk007.h18 (SEQ ID NO:4). The top row indicates with asterisks (\*) the amino acids conserved among both sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 3 depicts the alignment between the IT-2 precursor amino acid sequence from *Hottentotta judaica* (NCBI General Identifier No. 134344; SEQ ID NO:23) and the amino acid sequence derived from the instant *Buthotus judaicus* clone ibjlc.pk008.c4 (SEQ ID NO:6). The top row indicates with asterisks (\*) the amino acids conserved in both sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 4 depicts the alignment between the TsnTxp amino acid sequence from *Tityus serrulatus* (NCBI General Identifier No. 3293263; SEQ ID NO:24), the amino acid sequence derived from the instant *Buthotus judaicus* clones ibjlc.pk006.o21 (SEQ ID NO:8) and ibjlc.pk007.c5 (SEQ ID NO:10). The top row indicates with asterisks (\*) the amino acids conserved in all three sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 5 depicts the alignment between the insecticidal toxin Osl-1 amino acid sequence from *Orthochirus scrobiculosus* (NCBI General Identifier No. 3273105; SEQ ID NO:25) and the amino acid sequence derived from the instant *Buthotus judaicus* clone ibjlc.pk008.k24 (SEQ ID NO:12). The top row indicates with asterisks (\*) the amino acids conserved in both sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 6 depicts the alignment between the BmK M1 precursor amino acid sequence from *Mesobuthus martensii* (NCBI General Identifier No. 3047323; SEQ ID NO:26) with the amino acid sequences derived from the instant *Buthotus judaicus* clones ibjlc.pk007.f5 (SEQ ID NO:14), ibjlc.pk007.h1 (SEQ ID NO:16), and ibjlc.pk007.p23 (SEQ ID NO:18). The top row indicates with asterisks (\*) the amino acids conserved among all three sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 7 depicts the alignment between the neurotoxin V-5 amino acid sequence from *Centruroides exilicauda* (NCBI General Identifier No. 102790; SEQ ID NO:27) and the amino acid sequence deduced from the instant *Buthotus judaicus* clone ibjlc.pk005.k22 (SEQ ID NO:20). The top row indicates with asterisks (\*) the amino acids conserved in

both sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 8 depicts the alignment between the AS neurotoxin amino acid sequence from *Buthus martensii* (NCBI General Identifier No. 5712121; SEQ ID NO:38) and amino acid  
 5 sequence derived from the instant *Buthotus judaicus* clone ibj1c.pk006.f4 (SEQ ID NO:29). The top row indicates with asterisks (\*) the amino acids conserved in both sequences. The conserved cysteine residues probably involved in intrachain disulfide bridges are boxed.

Figure 9 depicts the alignment between the insecticidal toxin amino acid sequence from *Orthochirus scrobiculosus* (NCBI General Identifier No. 3273105; SEQ ID NO:25)  
 10 and the amino acid sequences derived from the instant *Buthotus judaicus* clones ibj1c.pk0004.h3 (SEQ ID NO:31), ibj1c.pk006.p4 (SEQ ID NO:33), ibj1c.pk008.f14 (SEQ ID NO:35), and ibj1c.pk008.l19 (SEQ ID NO:37). The top row indicates with asterisks (\*) that the amino acids are conserved among all sequences and with pluses (+) that the amino acids are conserved only among the *Buthotus judaicus* sequences. The conserved cysteine  
 15 residues probably involved in intrachain disulfide bridges are boxed.

The following sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

SEQ ID NO:1 is the nucleotide sequence comprising a substantial portion of the  
 20 cDNA insert in clone ibj1c.pk008.l9 encoding an entire *Buthotus judaicus* AAH IT4 with its entire signal sequence.

SEQ ID NO:2 is the deduced amino acid sequence of an entire *Buthotus judaicus* AAH IT4 with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:1. The mature toxin without its signal sequence consists of amino acids 21 through 86.

25 SEQ ID NO:3 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk007.h18 encoding an entire scorpion LqhIT2 precursor with its entire signal sequence.

SEQ ID NO:4 is the deduced amino acid sequence of an entire scorpion LqhIT2 precursor with its entire signal sequence derived from the nucleotide sequence of SEQ ID  
 30 NO:3. The mature toxin without its signal sequence consists of amino acids 22 through 84.

SEQ ID NO:5 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk008.c4 encoding an entire *Buthotus judaicus* IT-2 precursor with its entire signal sequence.

35 SEQ ID NO:6 is the deduced amino acid sequence of an entire *Buthotus judaicus* IT-2 precursor with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:5. The mature toxin without its signal sequence consists of amino acids 20 through 82.

SEQ ID NO:7 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibjlc.pk006.o21 encoding an entire *Buthotus judaicus* TsnTxp with its entire signal sequence.

5 SEQ ID NO:8 is the deduced amino acid sequence of an entire *Buthotus judaicus* TsnTxp with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:7. The mature toxin without its signal sequence consists of amino acids 19 through 82.

SEQ ID NO:9 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibjlc.pk007.c5 encoding an entire *Buthotus judaicus* TsnTxp with its entire signal sequence.

10 SEQ ID NO:10 is the deduced amino acid sequence of an entire *Buthotus judaicus* TsnTxp with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:9. The mature toxin without its signal sequence consists of amino acids 22 through 83.

SEQ ID NO:11 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibjlc.pk008.k24 encoding an almost entire *Buthotus judaicus* Osl-1 with a portion of its signal sequence.

15 SEQ ID NO:12 is the deduced amino acid sequence of an almost entire *Buthotus judaicus* Osl-1 with a portion of its signal sequence derived from the nucleotide sequence of SEQ ID NO:11. The mature toxin without its signal sequence consists of amino acids 19 through 80.

20 SEQ ID NO:13 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibjlc.pk007.f5 encoding an entire *Buthotus judaicus* BmK M1 precursor with its entire signal sequence.

SEQ ID NO:14 is the deduced amino acid sequence of an entire *Buthotus judaicus* BmK M1 precursor with its entire signal sequence derived from the nucleotide sequence of  
25 SEQ ID NO:13. The mature toxin without its signal sequence consists of amino acids 20 through 82.

SEQ ID NO:15 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibjlc.pk007.h1 encoding an entire *Buthotus judaicus* BmK M1 precursor with its entire signal sequence.

30 SEQ ID NO:16 is the deduced amino acid sequence of an entire *Buthotus judaicus* BmK M1 precursor with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:15. The mature toxin without its signal sequence consists of amino acids 24 through 84.

35 SEQ ID NO:17 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibjlc.pk007.p23 encoding an entire *Buthotus judaicus* BmK M1 precursor with its entire signal sequence.

SEQ ID NO:18 is the deduced amino acid sequence of an entire *Buthotus judaicus* BmK M1 precursor with its entire signal sequence derived from the nucleotide sequence of



SEQ ID NO:17. The mature toxin without its signal sequence consists of amino acids 20 through 83.

5 SEQ ID NO:19 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk005.k22 encoding an entire *Buthotus judaicus* neurotoxin V-5 with a portion of its signal sequence.

SEQ ID NO:20 is the deduced amino acid sequence of an entire *Buthotus judaicus* neurotoxin V-5 with a substantial portion of its signal sequence derived from the nucleotide sequence of SEQ ID NO:19. The mature toxin without its signal sequence consists of amino acids 21 through 94.

10 SEQ ID NO:21 is the amino acid sequence of the *Androctonus australis hector* AAH IT4 having the NCBI General Identifier No. 134360.

SEQ ID NO:22 is the amino acid sequence of the *Leiurus quinquestriatus hebraeus* LqhIT2 precursor having the NCBI General Identifier No. 1078960.

15 SEQ ID NO:23 is the amino acid sequence of the *Hottentotta judaica* IT-2 precursor having the NCBI General Identifier No. 134344.

SEQ ID NO:24 is the amino acid sequence of the *Tityus serrulatus* TsnTxp having the NCBI General Identifier No. 3293263.

SEQ ID NO:25 is the amino acid sequence of the *Orthochirus scrobiculosus* insecticidal toxin Osl-1 having the NCBI General Identifier No. 3273105.

20 SEQ ID NO:26 is the amino acid sequence of the *Mesobuthus martensii* BmK M1 precursor having the NCBI General Identifier No. 3047323.

SEQ ID NO:27 is the amino acid sequence of the *Centruroides exilicauda* neurotoxin V-5 having the NCBI General Identifier No. 102790.

25 SEQ ID NO:28 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk006.f4 encoding an entire AS neurotoxin with its entire signal sequence.

SEQ ID NO:29 is the deduced amino acid sequence of an entire *Buthotus judaicus* AS neurotoxin with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:28. The mature toxin without its signal sequence consists of amino acids 22 through 82.

30 SEQ ID NO:30 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk0004.h3 encoding an entire insecticidal toxin with its entire signal sequence.

35 SEQ ID NO:31 is the deduced amino acid sequence of an entire *Buthotus judaicus* insecticidal toxin with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:30. The mature toxin without its signal sequence consists of amino acids 22 through 89.

SEQ ID NO:32 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk006.p4 encoding an entire insecticidal toxin with its entire signal sequence.

5 SEQ ID NO:33 is the deduced amino acid sequence of an entire *Buthotus judaicus* insecticidal toxin with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:32. The mature toxin without its signal sequence consists of amino acids 22 through 89.

10 SEQ ID NO:34 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk008.f14 encoding an entire insecticidal toxin with its entire signal sequence.

SEQ ID NO:35 is the deduced amino acid sequence of an entire *Buthotus judaicus* insecticidal toxin with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:34. The mature toxin without its signal sequence consists of amino acids 22 through 89.

15 SEQ ID NO:36 is the nucleotide sequence comprising a substantial portion of the cDNA insert in clone ibj1c.pk008.119 encoding an entire insecticidal toxin with its entire signal sequence.

20 SEQ ID NO:37 is the deduced amino acid sequence of an entire *Buthotus judaicus* insecticidal toxin with its entire signal sequence derived from the nucleotide sequence of SEQ ID NO:36. The mature toxin without its signal sequence consists of amino acids 22 through 89.

SEQ ID NO:38 is the amino acid sequence of the *Buthus martensii* AS neurotoxin having the NCBI General Identifier No. 5712121.

25 The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

30 DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 60

contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 30 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36, or the complement of such sequences.

“NPV” stands for nuclear polyhedrosis virus, a baculovirus. “Polyhedrosis” refers to any of several viral diseases of insect larvae characterized by dissolution of tissues and accumulation of polyhedral granules in the resultant fluid. “PIBs” are polyhedral inclusion bodies. “AcNPV” stands for the wild-type *Autographa californica* nuclear polyhedrosis virus.

The terms “Na-channel modifier”, “Na-channel agonist”, “sodium-channel modifier”, and “sodium-channel agonist” are used interchangeably herein.

The term “isolated polynucleotide” refers to a polynucleotide that is substantially free from other nucleic acid sequences such as, and not limited to, other chromosomal and extrachromosomal DNA and RNA. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term “recombinant” means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic acids by genetic engineering techniques.

As used herein, “substantially similar” refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms “substantially similar” and “corresponding substantially” are used interchangeably herein.

For example, alterations in a gene which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded protein, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in the substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or

DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) *Nucleic Acid Hybridisation*, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS which was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-

based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively  
5 identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or  
10 more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that  
15 comprise one or more particular arthropod proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

20 "Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited  
25 by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building  
30 blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments  
35 may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan

appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign gene” refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in a variety of cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences

have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

“Translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

“3' Non-coding sequences” refers to nucleotide sequences located downstream of a coding sequence and includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and can be translated into polypeptides by the cell. “cDNA” refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. “Sense RNA” refers to an RNA transcript that includes the mRNA and can be translated into a polypeptide by the cell. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term “operably linked” refers to the association of two or more nucleic acid fragments so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. “Expression” may also refer to translation of mRNA into a polypeptide. “Overexpression” refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms.

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide. Each protein or polypeptide has a unique function.

5 "Altered levels" or "altered expression" refer to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

A "signal sequence" is an amino acid sequence that is covalently linked to an amino acid sequence representing a mature protein. The signal sequence directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53).

10 "Mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides, including signal sequences, present in the primary translation product have been removed. "Precursor protein" refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

15 "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation  
20 technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a  
25 polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., *Cloning Vectors: A Laboratory Manual*, 1985, supp. 1987; Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Flevin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990.  
30 Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a  
35 ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

It is understood that "an insect cell" refers to one or more insect cells maintained *in vitro* as well as one or more cells found in an intact, living insect.



Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

- 5 "PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

- 10 Nucleic acid fragments encoding at least a substantial portion of several scorpion Na-channel agonists have been isolated and identified by comparison of random arthropod cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. Table 1 lists the proteins that are described herein, and the designation of the cDNA clones that comprise the nucleic acid fragments encoding these proteins.

15

TABLE 1  
Sodium Channel Modifiers From  
*Buthotus judaicus* Telsons 48-Hours Post Milking

Enzyme	Clone
AAH IT4	ibj1c.pk008.i9
LqhIT2 Precursor	ibj1c.pk007.h18
IT-2 Precursor	ibj1c.pk008.c4
TsnTxp	ibj1c.pk006.o21
TsnTxp	ibj1c.pk007.c5
OsI-1	ibj1c.pk008.k24
BmK M1 Precursor	ibj1c.pk007.f5
BmK M1 Precursor	ibj1c.pk007.h1
BmK M1 Precursor	ibj1c.pk007.p23
Neurotoxin V-5	ibj1c.pk005.k22
AS Neurotoxin	ibj1c.pk006.f4
Insecticidal Toxin	ibj1c.pk0004.h3
Insecticidal Toxin	ibj1c.pk006.p4
Insecticidal Toxin	ibj1c.pk008.f14
Insecticidal Toxin	ibj1c.pk008.i19

- 20 The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other arthropod species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses

of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other AAH IT4s, LqhIT2 precursors, IT-2 precursors, TsnTxps, insecticidal toxins, BmK M1 precursors, neurotoxin V-5s, or AS neurotoxins, either as cDNAs or genomic DNAs, could be isolated directly by using all or a substantial portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired arthropod employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequence(s) can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding arthropod genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a scorpion Na-channel agonist polypeptide, preferably a substantial portion of an arthropod AAH IT4, LqhIT2 precursor, IT-2 precursor, TsnTxp, insecticidal toxin, BmK M1 precursor, neurotoxin V-5, or AS neurotoxin polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an AAH IT4, an LqhIT2 precursor, an IT-2 precursor, a TsnTxp, an insecticidal toxin, a BmK M1 precursor, neurotoxin V-5, or AS neurotoxin.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing substantial portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the chimeric genes of the invention as described herein or an isolated polynucleotide of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, plants, mammals and insects.

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed Na-channel agonists are expressed. This would be useful as a means for controlling insect pests by producing plants that are more insect-tolerant than the naturally occurring variety.

Expression in plants of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the

plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, LC-MS, or phenotypic analysis.

The instant polypeptides (or substantial portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded scorpion Na-channel agonist. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 8).

Insecticidal baculoviruses have great potential to provide an environmentally benign method for agricultural insect pest control. However, improvements to efficacy are required in order to make these agents competitive with current chemical pest control agents. One approach for making such improvements is through genetic alteration of the virus. For instance, it may be possible to modify the viral genome in order to improve the host range of the virus, to increase the environmental stability and persistence of the virus, or to improve the infectivity and transmission of the virus. In addition, improving the rate at which the virus acts to compromise the infected insect would significantly enhance the attractiveness of insecticidal baculoviruses as adjuncts or replacements for chemical pest control agents. One method for increasing the speed with which the virus affects its insect host is to introduce into the baculovirus foreign genes that encode proteins that are toxic to the insect wherein death or incapacitation of the insect is no longer dependent solely on the course of the viral infection, but instead is aided by the accumulation of toxic levels of the foreign protein. The results are insecticidal recombinant baculoviruses.

Recombinant baculoviruses expressing the instant scorpion Na-channel agonists (or substantial portions thereof) may be prepared by protocols now known to those skilled in the art (e.g., Tomalski et al., U.S. Patent No. 5,266,317, exemplifying neurotoxins from the insect-parasitic mites; McCutchen et al. (1991) *Bio/Technology* 9:848-852; Maeda et al.

(1991) *Virology* 184:777-780, illustrating construction of a recombinant baculovirus expressing AaIT; also see O'Reilly et al. (1992) *Baculovirus Expression Vectors: A Laboratory Manual*, W. H. Freeman and Company, New York; King and Possee (1992) *The Baculovirus Expression System*, Chapman and Hall, London; U.S. Patent No. 4,745,051).

- 5 These methods of gene expression provide economical preparation of foreign proteins in a eukaryotic expression vector system, in many instances yielding proteins that have achieved their proper tertiary conformation and formed the proper disulfide bridges necessary for activity.

Commonly, the introduction of heterologous genes into the baculovirus genome occurs  
10 by homologous recombination between viral genomic DNA and a suitable "transfer vector" containing the heterologous gene of interest. These transfer vectors are generally plasmid DNAs that are capable of autonomous replication in bacterial hosts, affording facile genetic manipulation. Baculovirus transfer vectors also contain a genetic construct comprising a region of the viral genome that has been modified to include the following features (listed in  
15 the 5' to 3' direction): 1) viral DNA comprising the 5' region of a non-essential genomic region; 2) a viral promoter; 3) one or more DNA sequences encoding restriction enzyme sites facilitating insertion of heterologous DNA sequences; 4) a transcriptional termination sequence; and 5) viral DNA comprising the 3' region of a non-essential genomic region. A heterologous gene of interest is inserted into the transfer vector at the restriction site  
20 downstream of the viral promoter. The resulting construct comprises a chimeric gene wherein the heterologous gene is under the transcriptional control of the viral promoter and transcription termination sequences present on the transfer vector. Moreover, this chimeric gene is flanked by viral DNA sequences that facilitate homologous recombination at a non-essential region of the viral genome. Recombinant viruses are created by co-transfecting  
25 insect cells that are capable of supporting viral replication with viral genomic DNA and the recombinant transfer vector. Homologous recombination between the flanking viral DNA sequences present on the transfer vector and the homologous sequences on the viral genomic DNA takes place and results in insertion of the chimeric gene into a region of the viral genome that does not disrupt an essential viral function. The infectious recombinant virion  
30 consists of the recombined genomic DNA, referred to as the baculovirus expression vector, surrounded by a protein coat.

In a preferred embodiment, the non-essential region of the viral genome that is present on the transfer vector comprises the region of the viral DNA responsible for polyhedrin production. Most preferred is a transfer vector that contains the entire polyhedrin gene  
35 between the flanking sequences that are involved in homologous recombination. Recombination with genomic DNA from viruses that are defective in polyhedrin production (due to a defect in the genomic copy of the polyhedrin gene) will result in restoration of the

polyhedrin-positive phenotype. This strategy facilitates identification and selection of recombinant viruses.

In another embodiment, baculoviral genomic DNA can be directly modified by introduction of a unique restriction enzyme recognition sequence into a non-essential region of the viral genome. A chimeric gene comprising the heterologous gene to be expressed by the recombinant virus and operably linked to regulatory sequences capable of directing gene expression in baculovirus-infected insect cells, can be constructed and inserted directly into the viral genome at the unique restriction site. This strategy eliminates both the need for construction of transfer vectors and reliance on homologous recombination for generation of recombinant viruses. This technology is described by Ernst et al. (Ernst et al. (1994) *Nuc. Acid Res.* 22: 2855-2856), and in WO 94/28114.

Recombinant baculovirus expression vectors suitable for delivering genetically encoded insect-specific neurotoxins require optimal toxin gene expression for maximum efficacy. A number of strategies can be used by the skilled artisan to design and prepare recombinant baculoviruses wherein toxin gene expression results in sufficient quantities of toxin produced at appropriate times during infection in a functional form and available for binding to target cells within the insect host.

The isolated toxin gene fragment may be digested with appropriate enzymes and may be inserted into the pTZ-18R plasmid (Pharmacia, Piscataway, NJ) at the multiple cloning site using standard molecular cloning techniques. Following transformation of *E. coli* DH5 $\alpha$ MCR, isolated colonies may be chosen and the plasmid DNA prepared. Positive clones will be identified and sequenced with the commercially available forward and reverse primers.

*Spodoptera frugiperda* cells (Sf-9) may be propagated in ExCellII<sup>®</sup> 401 media (JRH Biosciences, Lenexa, KS) supplemented with 3.0% fetal bovine serum. Lipofectin<sup>®</sup> (50  $\mu$ L at 0.1 mg/mL, Gibco/BRL) may be added to a 50  $\mu$ L aliquot of the transfer vector containing the toxin gene of interest (500 ng) and linearized polyhedrin-negative AcNPV (2.5  $\mu$ g, Baculogold<sup>®</sup> viral DNA, Pharmingen, San Diego, CA). Sf-9 cells (approximate 50% monolayer) may be co-transfected with the viral DNA/transfer vector solution. The supernatant fluid from the co-transfection experiment may be collected at 5 days post-transfection and recombinant viruses may be isolated employing standard plaque purification protocols, wherein only polyhedrin-positive plaques will be selected (Granados, R. R., Lawler, K. A., *Virology* (1981), 108, 297-308).

To propagate the recombinant virus of interest, isolated plaques may be picked and suspended in 500  $\mu$ L of ExCell<sup>®</sup> media supplemented with 2.5% fetal bovine serum. Sf-9 cells in 35 mM petri dishes (50% monolayer) may be inoculated with 100  $\mu$ L of the viral suspension, and supernatant fluids collected at 5 days post infection. These supernatant fluids will be used to inoculate cultures for large scale propagation of recombinant viruses.

Expression of the encoded toxin gene by the recombinant baculovirus will be confirmed using a bioassay, LCMS, or antibodies. The presence of toxin activity in the recombinant viruses will be monitored *in vivo*. These assays involve comparison of biological activity of recombinant viruses to wild-type viruses. Third instar larvae of  
5 *H. virescens* are infected orally by consumption of diet that contains test and control viruses and the larvae monitored for behavioral changes and mortality.

Isolated plugs of a standard insect diet are inoculated with approximately 5000 PIBs of each virus. Individual larvae that have not fed for 12 h prior to beginning of the bioassay are allowed to consume the diet for 24 h. The larvae are transferred to individual wells in a diet  
10 tray and monitored for symptoms and mortality on a daily basis (Zlotkin et al. (1991) *Biochimie (Paris)* 53:1073-1078).

### EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be  
15 understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

#### 20 EXAMPLE 1

##### Composition of cDNA Libraries: Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from *Buthotus judaicus* telsons were prepared. cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-  
25 ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England  
30 Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified  
35 insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

## EXAMPLE 2

### Identification of cDNA Clones

ESTs encoding Na-channel agonists were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also  
5 www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly  
10 available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience,  
15 the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

## EXAMPLE 3

### Characterization of cDNA Clones Encoding Na-Channel Agonists

The BLASTX search using the EST sequence from clone ibj1c.pk008.19 revealed similarity of the protein encoded by the cDNA to AAH IT4 from *Androctonus australis hector* (NCBI General Identifier No. 134360; pLog = 23.52). The sequence of a substantial  
25 portion of the cDNA insert from clone ibj1c.pk008.19 is shown in SEQ ID NO:1; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:2. This amino acid sequence contains a signal sequence (amino acids 1-20) and a mature protein (amino acids 21-86). The amino acid sequence set forth in SEQ ID NO:2 was evaluated by BLASTP, yielding a pLog value of 23.52 versus the *Androctonus australis hector* sequence. Figure 1  
30 presents an alignment of the amino acid sequences set forth in SEQ ID NO:2 and the *Androctonus australis hector* sequence (SEQ ID NO:21). The amino acid sequence presented in SEQ ID NO:2 is 64.6% identical to the *Androctonus australis hector* sequence.

The BLASTX search using the EST sequence from clone ibj1c.pk007.h18 revealed similarity of the protein encoded by the cDNA to LqhIT2 precursor from *Leiurus quinquestriatus hebraeus* (NCBI General Identifier No. 1078960; pLog = 10.70). The  
35 sequence of a substantial portion of the cDNA insert from clone ibj1c.pk007.h18 is shown in SEQ ID NO:3; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:4. This amino acid sequence contains a signal sequence (amino acids 1-21) and a mature



protein (amino acids 22-84). The amino acid sequence set forth in SEQ ID NO:4 was evaluated by BLASTP, yielding a pLog value of 10.70 versus the *Leiurus quinquestriatus hebraeus* sequence. Figure 2 presents an alignment of the amino acid sequences set forth in SEQ ID NO:4 and the *Leiurus quinquestriatus hebraeus* sequence (SEQ ID NO:22). The amino acid sequence presented in SEQ ID NO:4 is 28.6% identical to the *Leiurus quinquestriatus hebraeus* sequence.

The BLASTX search using the EST sequence from clone ibj1c.pk008.c4 revealed similarity of the protein encoded by the cDNA to IT-2 precursor from *Hottentotta judaica* (NCBI General Identifier No. 134344; pLog = 13.44). The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk008.c4 is shown in SEQ ID NO:5; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:6. This amino acid sequence contains a signal sequence (amino acids 1-19) and a mature protein (amino acids 20-82). The amino acid sequence set forth in SEQ ID NO:6 was evaluated by BLASTP, yielding a pLog value of 13.44 versus the *Hottentotta judaica* sequence. Figure 3 presents an alignment of the amino acid sequences set forth in SEQ ID NO:6 and the *Hottentotta judaica* sequence (SEQ ID NO:23). The amino acid sequence presented in SEQ ID NO:6 is 31.7% identical to the *Hottentotta judaica* sequence.

The BLASTX search using the EST sequence from clones ibj1c.pk006.o21 and ibj1c.pk007.c5 revealed similarity of the protein encoded by the cDNA to TsnTxp from *Tityus serrulatus* (NCBI General Identifier No. 3293263; pLog = 10.40 and 13.52, respectively). The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk006.o21 is shown in SEQ ID NO:7; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:8. This amino acid sequence contains a signal sequence (amino acids 1-18) and a mature protein (amino acids 19-82). The amino acid sequence set forth in SEQ ID NO:8 was evaluated by BLASTP, yielding a pLog value of 10.40 versus the *Tityus serrulatus* sequence. The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk007.c5 is shown in SEQ ID NO:9; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:10. This amino acid sequence contains a signal sequence (amino acids 1-21) and a mature protein (amino acids 22-83). The amino acid sequence set forth in SEQ ID NO:10 was evaluated by BLASTP, yielding a pLog value of 13.52 versus the *Tityus serrulatus* sequence. Figure 4 presents an alignment of the amino acid sequences set forth in SEQ ID NO:8, SEQ ID NO:10 and the *Tityus serrulatus* sequence (SEQ ID NO:24). The amino acid sequence presented in SEQ ID NO:8 is 30.5% identical to the *Tityus serrulatus* sequence while the amino acid sequence presented in SEQ ID NO:10 is 37.3% identical to the *Tityus serrulatus* sequence.

The BLASTX search using the EST sequence from clone ibj1c.pk008.k24 revealed similarity of the protein encoded by the cDNA to insecticidal toxin OsI-1 from *Orthochirus scrobiculosus* (NCBI General Identifier No. 3273105; pLog = 19.00). The sequence of a

substantial portion of the cDNA insert from clone ibj1c.pk008.k24 is shown in SEQ ID NO:11; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:12. This amino acid sequence contains a signal sequence (amino acids 1-18) and a mature protein (amino acids 19-80). The amino acid sequence set forth in SEQ ID NO:12 was evaluated by BLASTP, yielding a pLog value of 19.00 versus the *Orthochirus scrobiculosus* sequence. Figure 5 presents an alignment of the amino acid sequences set forth in SEQ ID NO:12 and the *Orthochirus scrobiculosus* sequence (SEQ ID NO:25). The amino acid sequence presented in SEQ ID NO:12 is 50.0% identical to the *Orthochirus scrobiculosus* sequence.

The BLASTX search using the EST sequence from clones ibj1c.pk007.f5, ibj1c.pk007.h1 and ibj1c.pk007.p23 revealed similarity of the protein encoded by the cDNA to BmK M1 precursor from *Mesobuthus martensii* (NCBI General Identifier No. 3047323; pLog = 32.30, 24.00 and 22.15, respectively). The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk007.f5 is shown in SEQ ID NO:13; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:14. This amino acid sequence contains a signal sequence (amino acids 1-19) and a mature protein (amino acids 20-82). The amino acid sequence set forth in SEQ ID NO:14 was evaluated by BLASTP, yielding a pLog value of 32.22 versus the *Mesobuthus martensii* sequence. The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk007.h1 is shown in SEQ ID NO:15; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:16. This amino acid sequence contains a signal sequence (amino acids 1-23) and a mature protein (amino acids 24-84). The amino acid sequence set forth in SEQ ID NO:16 was evaluated by BLASTP, yielding a pLog value of 24.0 versus the *Mesobuthus martensii* sequence. The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk007.p23 is shown in SEQ ID NO:17; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:18. This amino acid sequence contains a signal sequence (amino acids 1-19) and a mature protein (amino acids 20-83). The amino acid sequence set forth in SEQ ID NO:18 was evaluated by BLASTP, yielding a pLog value of 22.05 versus the *Mesobuthus martensii* sequence. Figure 6 presents an alignment of the amino acid sequences set forth in SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and the *Mesobuthus martensii* sequence (SEQ ID NO:26). The amino acid sequence presented in SEQ ID NO:14 is 72.6% identical to the *Mesobuthus martensii* sequence, the amino acid sequence presented in SEQ ID NO:16 is 56.0% identical to the *Mesobuthus martensii* sequence, and the amino acid sequence presented in SEQ ID NO:18 is 47.0% identical to the *Mesobuthus martensii* sequence.

The BLASTX search using the EST sequence from clone ibj1c.pk005.k22 revealed similarity of the protein encoded by the cDNA to neurotoxin V-5 from *Centruroides exilicauda* (NCBI General Identifier No. 102790; pLog = 5.30). The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk005.k22 is shown in SEQ ID NO:19; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:20. This

amino acid sequence contains a signal sequence (amino acids 1-20) and a mature protein (amino acids 21-99). The amino acid sequence set forth in SEQ ID NO:20 was evaluated by BLASTP, yielding a pLog value of 5.30 versus the *Centruroides exilicauda* sequence.

Figure 7 presents an alignment of the amino acid sequences set forth in SEQ ID NO:20 and the *Centruroides exilicauda* sequence (SEQ ID NO:27). The amino acid sequence presented in SEQ ID NO:20 is 28.8% identical to the *Centruroides exilicauda* sequence.

The data in Table 2 presents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 and the sequences in the NCBI database (SEQ ID NOs:21, 22, 23, 24, 25, 26 and 27).

**TABLE 2**

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Na-Channel Agonists

Clone	SEQ ID NO.	NCBI General Identifier No.	Percent Identity
ibjlc.pk008.l9	2	134360	64.6
ibjlc.pk007.h18	4	1078960	28.6
ibjlc.pk008.c4	6	134344	31.7
ibjlc.pk006.o21	8	3293263	30.5
ibjlc.pk007.c5	10	3293263	37.3
ibjlc.pk008.k24	12	3273105	50.0
ibjlc.pk007.f5	14	3047323	72.6
ibjlc.pk007.h1	16	3047323	56.0
ibjlc.pk007.p23	18	3047323	47.0
ibjlc.pk005.k22	20	102790	28.8

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Pairwise alignment of the sequences was performed using the Clustal method of alignment (Higgins, D. G. and Sharp, P. M. (1989) *CABIOS*. 5:151-153) using the default parameters (ktuple = 1, gap penalty = 3, window = 5, diagonals saved = 5). The default parameters for multiple alignment of the sequences using the Clustal method were: gap penalty = 10, gap length penalty = 10. Sequence alignments, BLAST scores and probabilities indicate that the instant nucleic acid fragments encode ten distinct, full-length, scorpion sodium channel modifiers with entire or nearly entire signal sequences: one AAH IT4, one LqhIT2 precursor, one IT-2 precursor, two TsnTxp, one OsI-1, two BmK MI precursors and one neurotoxin V-5.

#### EXAMPLE 4

##### Characterization of a cDNA Clone Encoding AS Neurotoxin

Further analysis of the DuPont proprietary database allowed the identification of other Na-channel agonists. The BLASTX search using the EST sequence from clone

5 ibj1c.pk006.f4 revealed similarity of the protein encoded by the cDNA to AS neurotoxin from *Buthus martensii* (NCBI General Identifier No. 5712121; pLog = 6.72). The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk006.f4 is shown in SEQ ID NO:28; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:29. This amino acid sequence contains a signal sequence (amino acids 1-21) and a mature protein  
10 (amino acids 22-82). The amino acid sequence set forth in SEQ ID NO:29 was evaluated by BLASTP, yielding a pLog value of 11.00 versus the *Buthus martensii* sequence. Figure 8 presents an alignment of the amino acid sequences set forth in SEQ ID NO:29 and the *Buthus martensii* sequence (SEQ ID NO:38). The amino acid sequence presented in SEQ ID NO:29 is 36.6% identical to the *Buthus martensii* sequence.

15 Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Pairwise alignment of the sequences was performed using the Clustal method of alignment (Higgins, D. G. and Sharp, P. M. (1989) *CABIOS*. 5:151-153) using the default parameters (ktuple = 1, gap penalty = 3, window = 5, diagonals saved = 5).  
20 Sequence alignments, BLAST scores and probabilities indicate that the instant nucleic acid fragment encodes a full-length scorpion AS neurotoxin with the entire signal sequence.

#### EXAMPLE 5

##### Characterization of cDNA Clones Encoding Novel Insecticidal Toxins

The BLASTX search using the EST sequences from clones ibj1c.pk0004.h3,  
25 ibj1c.pk006.p4, ibj1c.pk008.f14, and ibj1c.pk008.119 revealed similarity of the protein encoded by the cDNA to insecticidal toxin from *Orthochirus scrobiculosus* (NCBI General Identifier No. 3273105; pLog = 5.54, 4.27, 4.28, and 4.31, respectively). The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk0004.h3 is shown in SEQ ID NO:30; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:31. This  
30 amino acid sequence contains a signal sequence (amino acids 1-21) and a mature protein (amino acids 22-89). The amino acid sequence set forth in SEQ ID NO:31 was evaluated by BLASTP, yielding a pLog value of 6.05 versus the *Orthochirus scrobiculosus* sequence. The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk006.p4 is shown in SEQ ID NO:32; the deduced amino acid sequence of this cDNA is shown in SEQ  
35 ID NO:33. This amino acid sequence contains a signal sequence (amino acids 1-21) and a mature protein (amino acids 22-89). The amino acid sequence set forth in SEQ ID NO:33 was evaluated by BLASTP, yielding a pLog value of 5.00 versus the *Orthochirus scrobiculosus* sequence. The sequence of a substantial portion of the cDNA insert from

clone ibj1c.pk008.f14 is shown in SEQ ID NO:34; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:35. This amino acid sequence contains a signal sequence (amino acids 1-21) and a mature protein (amino acids 22-89). The amino acid sequence set forth in SEQ ID NO:35 was evaluated by BLASTP, yielding a pLog value of 5.00 versus the

5 *Orthochirus scrobiculosus* sequence. The sequence of a substantial portion of the cDNA insert from clone ibj1c.pk008.l19 is shown in SEQ ID NO:36; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:37. This amino acid sequence contains a signal sequence (amino acids 1-21) and a mature protein (amino acids 22-89). The amino acid sequence set forth in SEQ ID NO:37 was evaluated by BLASTP, yielding a pLog value

10 of 5.00 versus the *Orthochirus scrobiculosus* sequence. Figure 8 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:31, 33, 35, and 37 and the *Orthochirus scrobiculosus* sequence (SEQ ID NO:25). There are no more than 10 amino acid differences between the sequences of the instant invention. It was surprising to find that even though these sequences only have 7 Cys residues, making them incapable of forming 4 disulfide

15 bridges like Na-channel agonists normally do, in bioassays they showed activity against lepidopteran.

The data in Table 3 presents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:31, 33, 35, and 37 and the *Orthochirus scrobiculosus* sequence NCBI General Identifier No. 3273105 (SEQ ID NO:25).

**TABLE 3**

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Insecticidal Toxin

Clone	SEQ ID NO.	Percent Identity to 3273105
ibj1c.pk0004.h3	31	29.7
ibj1c.pk006.p4	33	29.6
ibj1c.pk008.f14	35	26.6
ibj1c.pk008.l19	37	26.6

25 Sequence alignments and percent similarity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins, D. G. and Sharp, P. M. (1989) *CABIOS*. 5:151-153) using the default

30 parameters (gap penalty = 10, gap length penalty = 10). Sequence alignments, BLAST scores, and probabilities indicate that the instant nucleic acid fragments encode four distinct, full-length, scorpion insecticidal toxins with entire signal sequences.

### EXAMPLE 6

#### Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (Nco I or Sma I) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below.

Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes Nco I and Sma I and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb Nco I-Sma I fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sal I-Nco I promoter fragment of the maize 27 kD zein gene and a 0.96 kb Sma I-Sal I fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236)

which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from cauliflower mosaic virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene  
5 from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1  $\mu$ m in diameter) are coated with DNA using the following technique. Ten  $\mu$ g of plasmid DNAs are added to 50  $\mu$ L of a suspension of gold particles (60 mg per mL). Calcium chloride  
10 (50  $\mu$ L of a 2.5 M solution) and spermidine free base (20  $\mu$ L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200  $\mu$ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles  
15 resuspended in a final volume of 30  $\mu$ L of ethanol. An aliquot (5  $\mu$ L) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

20 For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of mercury (Hg). The macrocarrier is accelerated  
25 with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to  
30 fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of  
35 tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) *Bio/Technology* 8:833-839).

### EXAMPLE 7

#### Expression of Chimeric Genes in Dicot Cells

A seed-specific construct composed of the promoter and transcription terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean  
5 *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin construct includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG  
10 translation initiation codon), Sma I, Kpn I and Xba I. The entire construct is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment  
15 when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed construct.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar  
20 A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

25 Soybean embryogenic suspension cultures can be maintained in 35 mL of liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of  
30 particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A Du Pont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from cauliflower mosaic virus (Odell et al.  
35 (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed construct comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the



phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50  $\mu$ L of a 60 mg/mL 1  $\mu$ m gold particle suspension is added (in order): 5  $\mu$ L DNA (1  $\mu$ g/ $\mu$ L), 20  $\mu$ L spermidine (0.1 M), and 50  $\mu$ L  $\text{CaCl}_2$  (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400  $\mu$ L 70% ethanol and resuspended in 40  $\mu$ L of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five  $\mu$ L of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches of mercury (Hg). The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

#### EXAMPLE 8

##### Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC, Philadelphia, PA). Buffer and agarose contain 10 µg/mL ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°C. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

### EXAMPLE 9

#### Expression of Chimeric Genes in Insect Cells

The cDNAs encoding the instant polypeptides may be introduced into the baculovirus genome itself. For this purpose the cDNAs may be placed under the control of the polyhedron promoter, the IE1 promoter, or any other one of the baculovirus promoters. The cDNA, together with appropriate leader sequences is then inserted into a baculovirus transfer vector using standard molecular cloning techniques. Following transformation of *E. coli* DH5α, isolated colonies are chosen and plasmid DNA is prepared and is analyzed by

restriction enzyme analysis. Colonies containing the appropriate fragment are isolated, propagated, and plasmid DNA is prepared for cotransfection.

*Spodoptera frugiperda* cells (Sf-9) are propagated in ExCell® 401 media (JRH Biosciences, Lenexa, KS) supplemented with 3.0% fetal bovine serum. Lipofectin® (50 µL at 0.1 mg/mL, Gibco/BRL) is added to a 50 µL aliquot of the transfer vector containing the toxin gene (500 ng) and linearized polyhedrin-negative AcNPV (2.5 µg, Baculogold® viral DNA, Pharmingen, San Diego, CA). Sf-9 cells (approximate 50% monolayer) are co-transfected with the viral DNA/transfer vector solution. The supernatant fluid from the co-transfection experiment is collected at 5 days post-transfection and recombinant viruses are isolated employing standard plaque purification protocols, wherein only polyhedrin-positive plaques are selected (O'Reilly et al. (1992), *Baculovirus Expression Vectors: A Laboratory Manual*, W. H. Freeman and Company, New York.). Sf-9 cells in 35 mM petri dishes (50% monolayer) are inoculated with 100 µL of a serial dilution of the viral suspension, and supernatant fluids are collected at 5 days post infection. In order to prepare larger quantities of virus for characterization, these supernatant fluids are used to inoculate larger tissue cultures for large scale propagation of recombinant viruses. Expression of the instant polypeptides encoded by the recombinant baculovirus is confirmed by bioassay.

#### EXAMPLE 10

##### Activity of Encoded Peptides Against *Heliothis Virescens*

It has been shown that single amino acids residues are important for receptor binding and for biological activity of scorpion Na-channel toxins (Zilbergberg et al. (1997) *J. Biol. Chem.* 272:14810-14816). Some of the cDNAs described herein were cloned into baculoviruses and used to test for their activity against Lepidopteran. Clones were tested in one or two independent bioassays against *Heliothis virescens*.

The DNA encoding the peptides was assayed for the presence of internal Bgl II and/or Eco RI restriction sites. Restriction sites useful for inserting the DNA fragments into the vector were added at the same time the DNA was amplified by using PCR. A Bgl II site was added at the 5' end of the DNA and an Eco RI site or an Asc I site (in clones containing internal Eco RI restriction sites) was added at the 3' end. Amplified DNA was inserted into the baculovirus transfer vector pAcUW21 (BD Biosciences-PharMingen, San Diego, CA). After amplification in *E. coli* the presence of the appropriate fragments was confirmed by restriction enzyme analysis. Colonies containing the appropriate fragments were isolated, propagated, and plasmid DNA was prepared for lipofectin-mediated co-transfection into insect cells with linearized polyhedrin-negative AcNPV. Co-transfections were performed essentially as described in Example 9. Polyhedrin-positive recombinant viruses were isolated employing standard plaque purification protocols and were mixed with a plug of HV diet (www.Bio-Serv.com) and fed to *Heliothis virescens* larvae.

Depicted in Table 4 are the results from two independent experiments in which four 5-day-old larvae were fed with 200 mg of viral-contaminated diet. The larvae were allowed to eat for 2 days or until the viral-contaminated diet was consumed, then fresh 1 g diet plugs were added to allow continued feeding. Larvae were examined for symptoms at 4, 5, 6, and 7 days after the fresh diet was added and scored as extremely active if most larvae showed toxic symptoms (paralysis) and died within 4 days, very active if death occurred within 5 days, active if death occurred within 6 days, and moderately active if a majority of the larvae presented toxic symptoms within 7 days, active if the larvae became irritated and had contractions and died and slightly active if the larvae had low diet consumption and a retardation in growth. These assays were compared to the results obtained by feeding insects with a diet containing wild-type AcNPV where the larvae die from melt-down after 7 days and by feeding insects with a control diet (water added instead of virus) where all the larvae survive.

TABLE 4

Activity of Scorpion Sodium Channel Modifiers  
on *Heliothis virescens* Larvae

Clone	SEQ ID NO:	Experiment 1	Experiment 2
ibj1c.pk008.l9	2	-	Extremely Active
ibj1c.pk007.h18	4	-	-
ibj1c.pk008.c4	6	-	Slightly Active
ibj1c.pk006.o21	8	-	-
ibj1c.pk007.c5	10	-	-
ibj1c.pk008.k24	12	-	-
ibj1c.pk007.f5	14	Active	Active
ibj1c.pk007.h1	16	Active	Active
ibj1c.pk007.p23	18	-	Very Active
ibj1c.pk005.k22	20	Active	Active
ibj1c.pk006.f4	29	Slightly Active	Slightly Active
ibj1c.pk0004.h3	31	Active	-
ibj1c.pk006.p4	33	Active	-
ibj1c.pk008.f14	35	Active	-
ibj1c.pk008.l19	37	Active	-

- Indicates that no bioassay was performed at this time.

In summary, the peptides encoded by the scorpion sequences depicted in SEQ ID NOs:2, 6, 14, 16, 18, 20, 29, 31, 33, 35, and 37 showed different levels of toxic activity against the lepidopteran *Heliothis virescens*.

It was an unexpected result to find that the peptides depicted in SEQ ID NOs:31, 33, 35, and 37 showed activity against lepidopteran although they only have 7 cysteine residues. The peptides may hold their three-dimensional structure by a different set of interactions.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
  - 5 (a) a nucleotide sequence of at least 180 nucleotides selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36;
  - (b) a nucleotide sequence encoding a polypeptide of at least 60 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 10 20, 29, 31, 33, 35, and 37; and
  - (c) a nucleotide sequence comprising a complement of (a) or (b).
2. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence of the fragment encodes a mature protein.
3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are 15 DNA.
4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.
5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.
- 20 6. A host cell comprising the chimeric gene of Claim 5.
7. A host cell comprising the isolated polynucleotide of Claim 1.
8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of yeast, bacteria, plant, mammals and insects.
9. A virus comprising the isolated polynucleotide of Claim 1.
- 25 10. A polypeptide of at least 60 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 29, 31, 33, 35, and 37.
11. A method of obtaining a nucleic acid fragment encoding a scorpion Na-channel agonist polypeptide comprising the steps of:
  - 30 (a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36 and a complement of such nucleotide sequences; and
  - (b) amplifying a nucleic acid sequence using the oligonucleotide primer.
- 35 12. A method of obtaining a nucleic acid fragment encoding a scorpion Na-channel agonist polypeptide comprising the steps of:
  - (a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence

selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 28, 30, 32, 34, and 36 and a complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

(c) isolating the identified DNA clone; and

5 (d) sequencing a cDNA or genomic fragment that comprises the isolated DNA clone.

13. A composition comprising the isolated polynucleotide of Claim 1.

14. A composition comprising the isolated polypeptide of Claim 10.

10 15. The isolated polynucleotide of Claim 1 comprising a nucleotide sequence having at least one of 30 contiguous nucleotides.

16. A recombinant baculovirus expression vector comprising a DNA sequence encoding a polypeptide of at least 60 amino acids comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 29, 31, 33, 35, and 37.

15 17. A method for testing the activity of a Na-channel agonist against insects comprising the steps of:

(a) amplifying a nucleic acid sequence;

(b) using restriction enzyme analysis to confirm an appropriate nucleic acid fragment is present;

20 (c) isolating the nucleic acid fragment;

(d) propagating colonies containing the isolated nucleic acid fragment;

(e) co-transfecting the isolated nucleic acid fragment into host cells with linearized polyhedrin-negative baculovirus;

(f) feeding larvae a viral-contaminated diet; and

25 (g) comparing a reaction of a viral contaminated larvae to a reaction of a non-viral contaminated larvae control group.

Figure 1

SEQ ID NO:21	*****	EHGYLLNKYTG	C	KVW	C	VINNEE	C	GYL	C	NKRRGGYYGY	C	YF
SEQ ID NO:02	-----	MMKIIIFLIVSSLMLIGVKSDDDGYLLNKATG	C	KVW	C	VNNAS	C	NSS	C	-KLRRGGNYGY	C	YF
												60

  

SEQ ID NO:21	****	WKLA	C	Y	C	QGARKSELWNYKTNK	C	D--L
SEQ ID NO:02	****	WKLA	C	Y	C	EGVAKSELWAYETNT	C	NGKL
								87



Figure 2

SEQ ID NO:22 MKLLLLIVSASMLIESLVNADGYIKRRDG [C] KVA [C] LIGNEG- [C] DKE [C] KAYGGSYGY [C] WTW  
 SEQ ID NO:04 MMKRILVLIAFSLVLIGADVNGYPKDSSG [C] KMT [C] ITGTDAL [C] NSI [C] KKLGGK-GE [C] Y-W  
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SEQ ID NO:22 GLA [C] W [C] EGLPDDKT-WKSETNT [C] GGKK  
 SEQ ID NO:04 GTI [C] W [C] TGVQNKDGLWDSNNNK [C] GGK-  
 61 87

### Figure 3

[illegible]

\*\* \* \* \* \* \*  
 SEQ ID NO:23 LA C W C ENLPD-AVTWKSSTNT C GRKK  
 SEQ ID NO:06 LA C W C DNIPNVPTWSYATNK C -RAK  
 61 86

### Figure 4

SEQ ID	NO:24	*	*	*	*	**	***	*	*	*	*	*	*	*	*	*	*	*	*
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SEQ ID	NO:10	MMKRILVLIAFSLVLIGADAHGYPKD-SKG	C	KMT	C	ITADDF	C	NSI	C	K-GIGGKGE	C	NW							

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	*	*	*	*	*	* * *	
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SEQ ID NO: 86	C	Y	C	TGMKDRSRLY-PGKNK	C	GGK	
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### Figure 5

[illegible]

SEQ ID NO:25 W C EGLPDN-KMWKYETNT C GGKK \*\*\*

SEQ ID NO:12 Y C EGMRDKSKLDRKTNK C GKG- 83

61

## Figure 9

[illegible]

	*    **	*	*	**	H-R
		C	W	IELPDNVPIRVPGK	C
SEQ ID NO: 26	WVGKYGNG	C	C	INLPDKVPIRIPGA	RGR
SEQ ID NO: 14	WLGLGYNA	C	C	INLPDKV--KEVK	RGG
SEQ ID NO: 16	WFGRYNA	C	C	VKLPEKTPIKS RGK	H-K
SEQ ID NO: 18	WFTPYGSV	C	C		89
	61				

[illegible]

Figure 8

SEQ ID NO:38	M-KTVIFLIVSSLLLLIGVKTDNGYLLDKYTG	C	KVW	C	VINNES	C	NSE	C	KIRGGYGY	C	YFW
SEQ ID NO:29	MMKIIIFLISLLMLIRVKNEGYLINVSKG	C	KVG	C	L-RSSF	C	YNE	C	KYPGGNGT	C	Y-W
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SEQ ID NO:38	KLA	C	F	C	QGARKSELWNYNTNK	C	NGKL
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[illegible]



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agacacgaac actgtagtaa cttttgtcaa tatttaggcg ctaaagggtgg taattgtaaa 180  
gatttcagtt gttactgtaa agcgcttcca aaaagtgtat cccataaatt agcagtaccg 240  
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<210> 20  
<211> 99  
<212> PRT  
<213> Hottentotta judaica

<400> 20  
Met Ser Lys Ile Phe Leu Ile Cys Thr Ala Leu Leu Leu Ile Asn Gly  
1 5 10 15  
Gln Val Glu Gly Thr Glu Thr Val Asp Ala Phe Pro Val Asn Asn Asn  
20 25 30  
Gly Cys Phe Tyr Pro Cys Tyr Ala Arg His Glu His Cys Ser Asn Phe  
35 40 45  
Cys Gln Tyr Leu Gly Ala Lys Gly Gly Asn Cys Lys Asp Phe Ser Cys  
50 55 60  
Tyr Cys Lys Ala Leu Pro Lys Ser Val Ser His Lys Leu Ala Val Pro  
65 70 75 80  
Trp Leu Phe Ser Cys Gly Thr Gly Tyr Leu Pro Asn Pro Thr Thr Thr  
85 90 95

Val Lys Pro

<210> 21  
<211> 65  
<212> PRT  
<213> Androctonus australis

<400> 21  
Glu His Gly Tyr Leu Leu Asn Lys Tyr Thr Gly Cys Lys Val Trp Cys  
1 5 10 15  
Val Ile Asn Asn Glu Glu Cys Gly Tyr Leu Cys Asn Lys Arg Arg Gly  
20 25 30  
Gly Tyr Tyr Gly Tyr Cys Tyr Phe Trp Lys Leu Ala Cys Tyr Cys Gln  
35 40 45  
Gly Ala Arg Lys Ser Glu Leu Trp Asn Tyr Lys Thr Asn Lys Cys Asp  
50 55 60

Leu  
65

<210> 22  
<211> 85  
<212> PRT  
<213> *Leiurus quinquestriatus*

<400> 22  
Met Lys Leu Leu Leu Leu Ile Val Ser Ala Ser Met Leu Ile Glu  
1 5 10 15  
Ser Leu Val Asn Ala Asp Gly Tyr Ile Lys Arg Arg Asp Gly Cys Lys  
20 25 30  
Val Ala Cys Leu Ile Gly Asn Glu Gly Cys Asp Lys Glu Cys Lys Ala  
35 40 45  
Tyr Gly Gly Ser Tyr Gly Tyr Cys Trp Thr Trp Gly Leu Ala Cys Trp  
50 55 60  
Cys Glu Gly Leu Pro Asp Asp Lys Thr Trp Lys Ser Glu Thr Asn Thr  
65 70 75 80  
Cys Gly Gly Lys Lys  
85

<210> 23  
<211> 85  
<212> PRT  
<213> *Hottentotta judaica*

<400> 23  
Met Lys Leu Leu Leu Leu Val Ile Ser Ala Ser Met Leu Leu Glu  
1 5 10 15  
Cys Leu Val Asn Ala Asp Gly Tyr Ile Arg Lys Lys Asp Gly Cys Lys  
20 25 30  
Val Ser Cys Ile Ile Gly Asn Glu Gly Cys Arg Lys Glu Cys Val Ala  
35 40 45  
His Gly Gly Ser Phe Gly Tyr Cys Trp Thr Trp Gly Leu Ala Cys Trp  
50 55 60  
Cys Glu Asn Leu Pro Asp Ala Val Thr Trp Lys Ser Ser Thr Asn Thr  
65 70 75 80  
Cys Gly Arg Lys Lys  
85

<210> 24  
<211> 84  
<212> PRT  
<213> *Tityus serrulatus*

<400> 24  
Met Lys Arg Met Ile Leu Phe Ile Ser Cys Leu Leu Leu Ile Asp Ile  
1 5 10 15  
Val Val Gly Gly Arg Glu Gly Tyr Pro Ala Asp Ser Lys Gly Cys Lys  
20 25 30  
Ile Thr Cys Phe Leu Thr Ala Ala Gly Tyr Cys Asn Thr Glu Cys Thr  
35 40 45

Leu Lys Lys Gly Ser Ser Gly Tyr Cys Ala Trp Pro Ala Cys Tyr Cys  
 50 55 60

Tyr Gly Leu Pro Asp Ser Val Lys Ile Trp Thr Ser Glu Thr Asn Lys  
 65 70 75 80

Cys Gly Lys Lys

<210> 25  
 <211> 64  
 <212> PRT  
 <213> Orthochirus scrobiculosus

<400> 25  
 Asp Gly Tyr Pro Lys Gln Lys Asp Gly Cys Lys Tyr Ser Cys Thr Ile  
 1 5 10 15

Asn His Lys Phe Cys Asn Ser Val Cys Lys Ser Asn Gly Gly Asp Tyr  
 20 25 30

Gly Tyr Cys Trp Phe Trp Gly Leu Ala Cys Trp Cys Glu Gly Leu Pro  
 35 40 45

Asp Asn Lys Met Trp Lys Tyr Glu Thr Asn Thr Cys Gly Gly Lys Lys  
 50 55 60

<210> 26  
 <211> 84  
 <212> PRT  
 <213> Mesobuthus martensi

<400> 26  
 Met Asn Tyr Leu Val Met Ile Ser Phe Ala Leu Leu Leu Met Thr Gly  
 1 5 10 15

Val Glu Ser Val Arg Asp Ala Tyr Ile Ala Lys Pro His Asn Cys Val  
 20 25 30

Tyr Glu Cys Ala Arg Asn Glu Tyr Cys Asn Asp Leu Cys Thr Lys Asn  
 35 40 45

Gly Ala Lys Ser Gly Tyr Cys Gln Trp Val Gly Lys Tyr Gly Asn Gly  
 50 55 60

Cys Trp Cys Ile Glu Leu Pro Asp Asn Val Pro Ile Arg Val Pro Gly  
 65 70 75 80

Lys Cys His Arg

<210> 27  
 <211> 59  
 <212> PRT  
 <213> Centruroides exilicauda

<400> 27  
 Lys Asp Gly Tyr Pro Val Asp Ser Lys Gly Cys Lys Leu Ser Cys Val  
 1 5 10 15

Ala Asn Asn Tyr Cys Asp Asn Gln Cys Lys Met Lys Lys Ala Ser Gly  
 20 25 30

Gly His Cys Tyr Ala Met Ser Cys Tyr Cys Glu Gly Leu Pro Glu Asn  
 35 40 45



Ala Lys Val Ser Asp Ser Ala Thr Asn Ile Cys  
50 55

<210> 28  
<211> 249  
<212> DNA  
<213> Hottentotta judiaca

<400> 28  
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ttttgttata acgaatgcaa atatccaggt ggtggtaatg ggacatgta ttggggattt 180  
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ggaaaaataa 249

<210> 29  
<211> 82  
<212> PRT  
<213> Hottentotta judiaca

<400> 29  
Met Met Lys Ile Ile Ile Phe Leu Ile Ser Leu Leu Leu Met Leu Ile  
1 5 10 15

Arg Val Lys Asn Glu Asp Gly Tyr Leu Ile Asn Val Ser Lys Gly Cys  
20 25 30

Lys Val Gly Cys Leu Arg Ser Ser Phe Cys Tyr Asn Glu Cys Lys Tyr  
35 40 45

Pro Gly Gly Gly Asn Gly Thr Cys Tyr Trp Gly Phe Cys Tyr Cys Thr  
50 55 60

Gly Met Lys Asp Arg Ser Arg Leu Tyr Pro Gly Lys Asn Lys Cys Gly  
65 70 75 80

Gly Lys

<210> 30  
<211> 270  
<212> DNA  
<213> Hottentotta judiaca

<400> 30  
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acagctttta atcataatta ttgtgtggac atttgtaaaag tacatggagt aaagtatggg 180  
tattgttggg tcacctcgtg ttgggtgtgaa tatttgaaaa aagaagacat cgatattttc 240  
aaagctatta aaaaccattg tagtaaataa 270

<210> 31  
<211> 89  
<212> PRT  
<213> Hottentotta judiaca

<400> 31  
Met Lys Ile Leu Val Leu Phe Gly Val Ile Leu Asn Leu Phe Tyr Leu  
1 5 10 15

Met Gly Ser Ile His Gly Gly Asp Thr Pro Gly Asn Tyr Pro Ile Ser  
20 25 30

Val Tyr Gly Thr Ser Tyr Gly Cys Thr Ala Phe Asn His Asn Tyr Cys  
35 40 45

Val Asp Ile Cys Lys Val His Gly Val Lys Tyr Gly Tyr Cys Trp Val  
50 55 60

Thr Ser Cys Trp Cys Glu Tyr Leu Lys Lys Glu Asp Ile Asp Ile Phe  
65 70 75 80

Lys Ala Ile Lys Asn His Cys Ser Lys  
85

<210> 32  
<211> 270  
<212> DNA  
<213> Hottentotta judiaca

<400> 32  
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acaagtagtt atcatgatta ttgtgcggac atttgtaaag tacatggagt gaattatggg 180  
tattgttggg tcacctcgtg ttgggtgtgaa tatttgaaag aagaagacat caatattttc 240  
gatgcactta aaaaccattg tagtaataaa 270

<210> 33  
<211> 89  
<212> PRT  
<213> Hottentotta judiaca

<400> 33  
Met Lys Ile Leu Val Leu Phe Gly Val Ile Leu Asn Leu Phe Tyr Leu  
1 5 10 15

Met Gly Ser Ile His Gly Gly Tyr Pro Pro Gly Asn Tyr Pro Ile Ser  
20 25 30

Ile Tyr Gly Lys Ser Tyr Gly Cys Thr Ser Ser Tyr His Asp Tyr Cys  
35 40 45

Ala Asp Ile Cys Lys Val His Gly Val Asn Tyr Gly Tyr Cys Trp Val  
50 55 60

Thr Ser Cys Trp Cys Glu Tyr Leu Lys Glu Glu Asp Ile Asn Ile Phe  
65 70 75 80

Asp Ala Leu Lys Asn His Cys Ser Lys  
85

<210> 34  
<211> 270  
<212> DNA  
<213> Hottentotta judiaca

<400> 34  
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acaagtagtg atcatgatta ttgtgcggac atttgtaaag tacatggagt gaattatggg 180  
tattgttggg tcacctcgtg ttgggtgtgaa tatttgaaag aagaagacat caatattttc 240  
gatgctgtta aaaaccattg tagtaataaa 270

<210> 35  
<211> 89  
<212> PRT  
<213> Hottentotta judiaca

<400> 35  
Met Lys Ile Leu Val Leu Phe Gly Val Ile Leu Asn Leu Phe Tyr Leu  
1 5 10 15

Met Gly Ser Ile His Gly Gly Tyr Pro Pro Gly Asn Tyr Pro Ile Ser  
                   20                  25                  30  
 Ile Tyr Gly Gln Ser Tyr Gly Cys Thr Ser Ser Asp His Asp Tyr Cys  
                   35                  40                  45  
 Ala Asp Ile Cys Lys Val His Gly Val Asn Tyr Gly Tyr Cys Trp Val  
                   50                  55                  60  
 Thr Ser Cys Trp Cys Glu Tyr Leu Lys Glu Glu Asp Ile Asn Ile Phe  
                   65                  70                  75                  80  
 Asp Ala Val Lys Asn His Cys Ser Lys  
                                   85

<210> 36  
 <211> 270  
 <212> DNA  
 <213> Hottentotta judiaca

<400> 36  
 atgaagatat tgggtgttatt tgggtgtcatc cttaacttat tctattttaat ggggttcaatt 60  
 catggaggct atccaccagg aaattaccg atatctattt atggtaagtc ttatggatgc 120  
 acaagtagtt atcatgatta ttgtgcggac atttgtaaag tacatggagt gaattatggg 180  
 tattgttggg tcacctcgtg ttgggtgtgaa tatttgaaag aagaagacat caatattttc 240  
 gatgcattta aaaaccattg tagtaataa 270

<210> 37  
 <211> 89  
 <212> PRT  
 <213> Hottentotta judiaca

<400> 37  
 Met Lys Ile Leu Val Leu Phe Gly Val Ile Leu Asn Leu Phe Tyr Leu  
           1                  5                  10                  15  
 Met Gly Ser Ile His Gly Gly Tyr Pro Pro Gly Asn Tyr Pro Ile Ser  
                   20                  25                  30  
 Ile Tyr Gly Lys Ser Tyr Gly Cys Thr Ser Ser Tyr His Asp Tyr Cys  
                   35                  40                  45  
 Ala Asp Ile Cys Lys Val His Gly Val Asn Tyr Gly Tyr Cys Trp Val  
                   50                  55                  60  
 Thr Ser Cys Trp Cys Glu Tyr Leu Lys Glu Glu Asp Ile Asn Ile Phe  
                   65                  70                  75                  80  
 Asp Ala Phe Lys Asn His Cys Ser Lys  
                                   85

<210> 38  
 <211> 85  
 <212> PRT  
 <213> Buthus martensii

<400> 38  
 Met Lys Thr Val Ile Phe Leu Ile Val Ser Ser Leu Leu Leu Ile Gly  
           1                  5                  10                  15  
 Val Lys Thr Asp Asn Gly Tyr Leu Leu Asp Lys Tyr Thr Gly Cys Lys  
                   20                  25                  30  
 Val Trp Cys Val Ile Asn Asn Glu Ser Cys Asn Ser Glu Cys Lys Ile  
                   35                  40                  45

Arg Gly Gly Tyr Tyr Gly Tyr Cys Tyr Phe Trp Lys Leu Ala Cys Phe  
50 55 60

Cys Gln Gly Ala Arg Lys Ser Glu Leu Trp Asn Tyr Asn Thr Asn Lys  
65 70 75 80

Cys Asn Gly Lys Leu  
85